bio-protocol Preprint

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Real-time PCR analysis

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An abbreviated version of this protocol was published in Science Translational Medicine in Mar 2020

A high-salt diet compromises antibacterial neutrophil responses through hormonal perturbation

DOI: 10.1126/scitranslmed.aay3850

Detailed protocol

Request a Protocol: "Real-time PCR analysis"

1. Collect samples to an RNase free 1,5mL tube containing an appropriate amount of RA1 buffer (according to NucleoSpin RNA KIT)
2. Isolate the RNA according to manufacturer's protocol
3. Elute RNA with 20µl H_2O and measure the concentration and quality via NanoDrop Spectrophotometer (ratio absorbance 260/280nm)
4. Use 1000ng of RNA per sample for cDNA synthesis (via cDNA reverse transcriptase KIT)

Material	Volume (µl)
10x RT Buffer	2
10x RT Primer	2
25x dNTP	0,8
reverse transcriptase	1
RNAse inhibitor	1

5. Perform reverse transcription under following conditions

Conditions	Time (min)
Step 1: 25°C	10
Step 2: 37°C	120
Step 3: 85°C	5
Step 4: 4°C	unlimited

6. Dilute cDNA 1:5 in DEPC water
7. Dilute the primers in the following way (inhouse designed, generated from Quiagen)
    a. add an appropriate amount of DEPC water to lyophilized primers to reach a 100uM stock solution
    b. dilute primers further 1:10 in DEPC water
8. Put 1µl of cDNA for each sample on a clear 384-well plate in triplicates
9. Add 9µl of Master Mix per well

Material	Volume (µl)
SYBR Green	5
Primer Mix	1
DEPEC water	3
cDNA	1

10. Seal the plate with a cover foil and insert the plate into the Light Cycler 480 machine
11. run RT-PCR under the following conditions

Conditions	Time (min)
Step 1: 50°C	2
Step 2: 95°C	10
Step 3: 60°C	1 (repeated 45 times)
Step 4: 4°C	unlimited

Analysis of raw data

1. Export the "Crossing Point" (CP-value) of each sample
2. Generate the average of each CP-value from triplicates
3. Calculate the relative Quantification via reference gene (GAPDH) of each sample

    □CP= 2^- (CP target gene – CP housekeeping gene)

4. Values can be normalized by setting the control group as 1 and calculating the relative changes

Material

Material	Company	Reference Number
NucleoSpin RNA KIT	Macherey-Nagel	740955.250
NanoDrop Spectrophotometer	Thermo Fisher	ND-2000
cDNA reverse transcriptase KIT	Thermo Fisher	4368814
384-well plate	Roche Diagnostics	04729749001
Light Cycler 480 machine	Roche Diagnostics	05015243001
SYBR Green PCR MasterMix	Thermo Fisher	A46012

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Stumpf, N. E.(2020). Real-time PCR analysis. Bio-protocol Preprint. bio-protocol.org/prep291.
2. Jobin, K., Stumpf, N. E., Schwab, S., Eichler, M., Neubert, P., Rauh, M., Adamowski, M., Babyak, O., Hinze, D., Sivalingam, S., Weisheit, C., Hochheiser, K., Schmidt, S. V., Meissner, M., Garbi, N., Abdullah, Z., Wenzel, U., HÃ¶lzel, M., Jantsch, J. and Kurts, C.(2020). A high-salt diet compromises antibacterial neutrophil responses through hormonal perturbation . Science Translational Medicine 12(536). DOI: 10.1126/scitranslmed.aay3850